656

Human facial sebaceous glands contain the enzymes that synthesise prostaglandin $F_{2\alpha}$ and prostamide $F_{2\alpha}$ and the receptors to respond to bimatoprost

M Shalbaf,[1] T Street,[1] NJ Poloso,[2] JW Wang,[2] D Woodward[2] and V Randall[1] *1 Centre for Skin Sciences, University of Bradford, Bradford, United Kingdom and 2 Departments of Biological and Chemical Sciences, Allergan Inc., Irvine, CA*

Acne, a common skin disorder, has a complex pathogenesis associated with androgen-stimulated increased sebum production, duct blockage, microbial infection and inflammation. Available treatments have significant limitations, signifying a need for novel therapies. Recently, bimatoprost, a prostamide $F_{2\alpha}$ analogue used for glaucoma and stimulating eyelash growth, has been shown to inhibit lipid production in human adipocytes. Prostamides are a new class of molecules closely related to prostaglandins. Since COX2, a prostaglandin (PG) synthesising enzyme, is raised in acne, we hypothesise that PG/prostamide signalling may be involved in its pathogenesis. We investigated whether human facial sebaceous glands contain receptors for prostamide $F_{2\alpha}$ and can synthesise $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ *de novo* from phospholipids. To identify gene expression, individual sebaceous glands were microdissected from female facial skin (n=5), RNA extracted and cDNA synthesised. Proteins were located by immunohistochemistry in frozen skin sections (n=5). RT-PCR and qPCR analyses revealed gene expression of 3 splice variants of $PGF_{2\alpha}$ receptor (FP), native FP and variants altFP1 and altFP4, and all the enzymes involved in both $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ synthesis (PLA2, COX1, COX2, FAAH1, FAAH2, PGFS, NAPE-PLD, and PM/PGFS). Immunohistochemistry located protein expression of native FP and the synthetic enzymes in sebaceous glands, predominantly in the basal sebocytes around the edges of the acini. Therefore, individual human facial sebaceous glands contain receptors for $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ and PG and prostamide synthesising enzymes indicating facial sebaceous glands should be able to respond to bimatoprost and to synthesise both $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ *de novo* from phospholipids. Further understanding of the roles of these signalling molecules in human sebaceous glands may lead to new acne therapies.

657

A comparison of methods of anagen synchronization in an animal model for alopecia

AC Villasante[1, 2], VA Petit,[2] N Yin[2, 3], G Elgart,[2] L Schachner[2] and J Jimenez[2] *1 Medical College, Weill Cornell Medical College, New York, NY, 2 Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL and 3 Dermatology, Columbia University, New York, NY*

Purpose: Anagen synchronization is an essential step in experiments on murine models for several hair disorders, including alopecia areata, androgenetic alopecia, and chemotherapy-induced alopecia (CIA). Methods commonly used to induce anagen in animal models include clipping, shaving, waxing, and plucking. Currently, there is no consensus as to which study is best. This study compares, for the first time, the effectiveness and side effects of these four methods in a rat model for CIA. Methods: Biopsies of adult rat skin were analyzed after treatment with one of four methods of anagen synchronization: clipping, shaving, waxing, or plucking. Anagen synchronization was induced on postnatal day 21 and histological analysis was performed on day 36. Twenty follicles were assessed at random in each group and the percentage of hair follicles in anagen V-VI was determined. Results: There were significant differences in the histological and gross profiles of clipping, shaving, waxing, and plucking. Clipping produced the most robust anagen, with 85% of hair follicles in anagen V-VI, while shaving was found to have 30% of follicles in anagen V-VI (p=0.001). Waxing, plucking, and control had no hair follicles in anagen V-VI. Furthermore, shaving, waxing, and plucking demonstrated noticeable gross skin trauma, with waxing resulting in the most severe. Conclusion: Clipping is the preferred method of anagen synchronization in the adult rat model for CIA, as it produces the most robust anagen and has no evidence of gross skin trauma, decreasing the likelihood of confounding results from traumatic hair removal.

658

Knockdown of *Sulf2* causes hair loss in obese mice fed a fast food diet

JM Olazagasti,[1] CD Moser,[2] TH Kim,[2] A Krishnan[2] and LR Roberts[2] *1 Mayo Graduate School, Mayo Clinic, Rochester, MN and 2 Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN*

Signaling molecules such as Wnts, bone morphogenic proteins (BMPs), sonic hedgehog (Shh), Notch, and platelet-derived growth factor (PDGF) play a key role in hair follicle development and cycling. These growth factors and morphogens are thought to be regulated by heparan sulfate proteoglycans (HSPGs), which act as storage sites for growth factors and as co-receptors in growth factor-receptor interactions. The enzymatic removal of 6-*O*-sulfate by sulfatase 2 (SULF2) releases growth factors from extracellular storage sites turning active multiple signaling pathways. Serendipitously, our studies of mice with a mutation in SULF2 suggest a role for this enzyme in regulation of hair follicle development. We observed that knockout of SULF2 causes hair loss in a mouse model of non-alcoholic steatohepatitis (NASH) induced by a fast food diet. SULF2-knockout mice on a fast food diet developed sharply demarcated and irregularly-shaped areas of complete hair loss. Histological analysis of skin biopsy specimens revealed normal hair follicles and a thickened intradermal adipose tissue. Recent findings suggest intradermal adipose tissue is closely related with hair growth. Adiposederived stem cells (ASCs) produce and secrete growth factors such as PDGF, which have been implicated in hair growth induction by activating the Wnt pathway. Furthermore, the number of ASCs changes with the hair cycle. ASC number peaks in the skin during the hair growth phase (anagen) and decreases during the apoptosis-driven involution of hair (catagen). On the other hand, mature adipocyte cells express BMP2, which is an inhibitory signal for regeneration of the hair follicle. The molecular regulatory network in hair development is complex and has not been completely elucidated. Our mouse model identifies SULF2 as an important regulator of hair growth and suggests that cellular mechanisms of its action involve the release of heparan sulfate-binding growth factors, thus regulating the hair cycle.

659

Follistatin and secreted frizzled-related protein 1, OVO homolog-like 1- regulated genes, are important for hair follicle neogenesis

S Bak, M Kim, J Kim and Y Sung *Kyungpook National University School of Medicine, Daegu, Korea (the Republic of)*

Identification of genes critical for hair follicle induction is of great importance for the development of alternative treatments for hair loss by cell implantation as well as for our understanding of hair follicle development. We, very recently, demonstrated that OVO homolog-like 1 (OVOL1) has a critical role in hair follicle neogenesis. Since OVOL1 itself is a transcription factor, in this study we proceed to investigate which OVOL-1 regulated gene(s) affect the potency of hair induction in dermal cells. Cultured neonatal dermal cells were transduced with either control lentivirus (Lt-control) or lentivirus carrying OVOL1 (Lt-OVOL1) and gene expression analysis were performed. A number of genes including follistatin (Fst) and secreted frizzled-related protein 1 (SFRP1) were differentially up-regulated in Lt-OVOL1 transduced dermal cells. We found that Fst siRNA-transfected dermal cells showed significantly impaired hair follicle induction. In addition, we found significant impairment of hair follicle induction by SFRP1 knockdown. Our data strongly suggest that OVOL1-inducible FST and SFRP1 are involved in hair follicle neogenesis.

660

Stimulatory effect of pseudoceramide on hair growth through intracellular sphingolipids signaling

JH Bae,[1] BM Park,[1] DH Kim,[1] KO Shin,[2] SP Hong,[3] HJ Kim,[4] SK Ahn[5] and S Jeong[1, 2] *1 Research Division, Neopharm, Daejeon, Korea (the Republic of), 2 Department of Pharmacy, Chungbuk National University, Cheongju, Korea (the Republic of), 3 Department of Dermatology, Dankook University College of Medicine, Cheonan, Korea (the Republic of), 4 Department of Dermatology, Atopy Clinic, Seoul Medical Center, Seoul, Korea (the Republic of) and 5 Department of Dermatology, Yonsei University Wonju College of Medicine, Wonju, Korea (the Republic of)*

Various factors including psychological and/or physical stress, endocrine dysfunction, and genetic predisposition result in a hair loss and its incidence is continuously increasing. Therefore, developing a new compound for the treatment of hair is of great importance. In the present study, hair growth promoting effects of newly synthesized pseudoceramide was evaluated by in vivo and in vitro models. In a mouse model, 200 of 1% pseudoceramide solution was topically applied on the dorsal skin of mice after shaving for 6 weeks. As a result, hair growing was significantly accelerated in the treated group, which might be, at least in part, due to the stimulation of hair anagen phase. In order to further investigate the underlying working mechanism of pseudoceramide, primary human dermal papillar cells (hDPCs) was treated with the compound for 8 and 24 hours and changes in intracellular sphingolipids species were analyzed. Increased amounts of ceramide, sphingosine, and shinganine were detected in hDPCs. Based on a previous report about the hair growth stimulating activity of topical sphinganine, it is postulated that sphingolipids modulating effects of pseudoceramide may result in an increased hair growth. Sphingolipids modulating effects of pseudoceramide was further confirmed by the accelerated recovery of epidermal permeability barrier function, evaluated by measuring the change of transepidermal water loss (TEWL) in acute barrier disruption model.

661

Systematic analyses for skin, hair, and nail abnormalities in The Jackson Laboratory's KOMP^2 knockout mouse program

JP Sundberg,[1] CH Pratt,[2] SA Murray,[2] KA Silva,[1] VE Kennedy,[1] LE King[3] and SS Dadras[4] *1 Research and Development, The Jackson Laboratory, Bar Harbor, ME, 2 Department of Genetic Resources Science, The Jackson Laboratory, Bar Harbor, ME, 3 Division of Dermatology, Vanderbilt University, Nashville, TN and 4 Departments of Dermatology and Genetics & Developmental Biology, University of Connecticut, Farmington, CT*

The development of genetically engineered mice (GEMs), initially by the insertion of a DNA construct from a number of sources/species into a mouse (transgenesis) and later by targeted gene deletion, has revolutionized biomedical research. The International Knockout Mouse Consortium (IKMC) was formed to inactivate ("knockout") all protein coding genes in the mouse genome in embryonic stem (ES) cells using a series of standardized targeted mutagenesis protocols and more recently the CRISPR/Cas9 technology. The Jackson Laboratory is one of three NIH-supported centers, generating more than 830 lines of live mice from these ES cell lines by the end of 2015. We now receive 2 female and 2 male heterozygous or homozygous retired breeder mice representing 10-15 novel lines per week. The resultant mutant mice are characterized initially by detailed histopathology of skin from multiple anatomic sites (dorsal and ventral skin, muzzle, ear, tail, eyelid, and feet including the entire nail unit). Whole organ system review is done on additional mice with lesions in any of the targeted anatomic sites. Representative photomicrographs (standard digital images and whole-slide scanned images) are placed on Mouse Genome Informatics. (http://www.informatics.jax.org/) and Pathbase (http://www.pathbase.net/) which are public access databases. An updated listing of lines screened will be presented.

662

Wnt/β-catenin signaling drives epidermal fate specification in human embryonic stem cells

S Lin, KA Rosowski, A Farrell, A Tadeu, C O'Curran and V Horsley *Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT*

Genetic skin diseases, chronic ulcers and severe burn wounds impair the regenerative process of the skin, leaving individuals susceptible to life-threatening infections. Tissue-based replacement therapies like autologous skin grafting are successful in approach but are insufficient for victims with extensive cutaneous lesions. Since human embryonic stem cells (hESCs) can undergo rapid expansion and be cued to differentiate into any somatic tissue, these cells are an excellent source for the derivation of allogeneic keratinocytes. However methods aimed to specify hESCs towards a keratinocyte fate remain highly inefficient. This is due to a lack in understanding of the molecular mechanisms that govern epidermal specification. The p63 transcription factor is essential for maintaining normal epidermal development and is required for murine ESCs to enter a keratinocyte fate. Gaining a better understanding of the molecular cues that control p63 may accelerate the derivation of keratinocytes from hESCs. To explore possible regulators of p63, we performed RNA-seq on undifferentiated and epidermal progenitors derived from hESCs. We found that the Wnt/β-catenin pathway represented one of the largest groups of genes to be altered during this process. We validated an increase in the transcriptional levels for several canonical Wnt target genes in hESCs triggered to undergo epidermal specification. Increases in Wnt/β-catenin activity occurred in synchrony with an upregulation in p63 transcript levels. To determine whether the Wnt pathway was required to regulate p63, hESCs were transduced with lentiviral constructs containing short hairpins targeted against β-catenin. This led to a reduction in p63 transcripts in epidermal specified hESCs. In contrast, activating the Wnt/β-catenin pathway by pharmacologically inhibiting GSK-3β led to an increase in p63 transcript levels and protein abundance. Taken together, this data demonstrates that a Wnt/β-catenin-p63 signaling axis may be targeted to enhance the formation of epidermal keratinocytes from hESCs.

663

Dynein is necessary for intracellular transport of both nutrients and autophagosomes in human dermal fibroblasts

Y Zhuang, R Wyborski, S Chen, RP Khusial and J Lyga *Avon Products Inc, Suffern, NY*

Cytoplasmic dynein is a molecular motor that is responsible for the movement of a wide range of cargoes along microtubules. It plays a role in numerous cellular processes including endosomal sorting, autophagy, cell division and cell migration. Dynein deficiency caused by genetic mutation has been linked to a number of neurodegenerative diseases. Disruption of axonal transport, a process that is mediated by dynein, is presented as a common feature occurring in neurodegenerative diseases. Dynein also plays a role in autophagy in neuronal cells since dynein is responsible for moving autophagosomes from cell periphery towards lysosomes. Given the importance of cytoplasmic dynein in neural system, we were interested in investigating the role it plays in skin cells. We investigated the impact of dyneins on transport of nutrients and autophagosomes in primary human dermal fibroblasts (HDF). In normal HDF cells, all autophagosomes were found at the central area of the cells and they showed perfect colocalization with lysosomes, which were also centrally located. This indicated that all autophagosomes were quickly transported to lysosomes and degraded there. In contrast, after we knocked down cytoplasmic dynein heavy chain 1 (DYNC1H1) in HDF cells, most autophagosomes were found retained at the cell periphery and little colocalization with lysosome was observed, indicating they were not being transported to lysosome for degradation/recycle. These observations were confirmed by using a known dynein inhibitor erythro-9-(2-Hydroxy-3-nonyl) adenine (EHNA). We also asked whether the nutrients transport to mitochondria was impaired in dynein knock-down HDFs. We found a reduction in nutrient transport to mitochondria in these cells when dynein was knocked down. In conclusion, our results indicate that dynein is essential for the well-being of human skin fibroblasts, by transporting essential nutrients to produce energy and degrading the waste materials. Therefore, boosting dynein level or activity in skin cells may provide skin cells better nourishment and better detoxification.

664

Dynamic interactions between nail epithelium and digit bone by Wnt signaling

M Takeo,[1] CS Hale[1, 2] and M Ito[1, 3] *1 Dermatology, New York University School of Medicine, New York, NY, 2 Pathology, New York University School of Medicine, New York, NY and 3 Cell Biology, New York University School of Medicine, New York, NY*

Clinically, many nail disorders accompany bone deformities. However whether or not the two defects are causally related is under debate. To investigate the potential interactions between the two tissue types, we analyzed epithelial-specific β-catenin deficient mice in which nail differentiation is abrogated. We found that these mice show regression of not only the nail epithelium but also the underlying digit bone, providing the first evidence that changes in nail epithelium can influence the maintenance of the underlying bone. Characterization of the bone defects revealed that osteoblasts lack Wnt signal activation, a signal known to be essential for their differentiation and ability to suppress bone resorption. Further, we found that Wntless expression, essential for Wnt ligand secretion, was lacking in β-catenin deficient nail epithelium and that genetic deletion of Wntless in the nail epithelium led to the down-regulation of Wnt activation in osteoblasts, which was rescued by the injection of canonical Wnt ligands into the digit tip, and defective regression of the underlying digit bone. Together, these data show how epithelial carnonical Wnt ligands can non-cell autonomously regulate Wnt signaling in osteoblasts. These results unveil a dynamic interplay between the nail epithelium and digit bone during homeostatic regeneration and show that Wnt/b-catenin signaling is key to this interaction.

665

Alterations of vitamin A metabolism and signaling in central, centrifugal, cicatricial alopecia patients

L Suo,[2] WF Bergfeld,[1] N Mesinkovska[1] and HB Everts[2] *1 Dermatology, Cleveland Clinic, Cleveland, OH and 2 Nutrition, Ohio State University, Columbus, OH*

Central, centrifugal, cicatricial alopecia, or CCCA is the most common scarring hair loss (alopecia) among African American women. Although the pathogenesis of CCCA is unknown, vitamin A plays an important role in the development of CCCA. Previously, in mice we found that many key components in vitamin A metabolism and signaling were altered in CCCA including: DHRS9 (dehydrogenase reductase member 9); ALDH1A1 (retinal dehydrogenase 1); CYP26A1 (cytochrome P450 26A1); and RARB (retinoic acid receptor beta). Their expression increased in mild disease and decreased in severe disease. The purpose of this study was to examine the possible alteration of those proteins among CCCA patients. Reviewed were African American women diagnosed with CCCA at Cleveland Clinic in the past eight years. These patients were initially clinically evaluated with a standardized central scalp alopecia photographic grading scale that graded the disease severity. The diagnosis of CCCA and severity was confirmed histologically. The control subjects were African American women diagnosed with pilar cyst. Totally, we had 11 mild disease, 11 moderate disease, 5 severe disease and 12 controls. Immunohistochemistry (IHC) on all the scalp biopsy samples using antibodies against DHRS9, ALDH1A1, CYP26A1 and RARB were examined. The results were a decrease of all four proteins ($p<0.01$) in the basal layer of the severe group as compared to controls. Also found was a decrease of RARB expression ($p<0.01$) in sweat glands and dermis in the severe group as compared to the mild group. These findings suggest that the expression of important vitamin A metabolism and signaling components in the skin decreases as severity increased. These findings expand the knowledge of pathogenesis of CCCA and emphasize the importance of vitamin A metabolism and signaling in the health of skin and hair.

666

Human hair follicle epithelial stem cells undergo epithelial-mesenchymal transition (EMT) in primary cicatricial alopecia: Lessons from lichen planopilaris

H Imanishi,[1] D Ansell,[1] M Harries,[1] N Sepp,[2] T Biro,[4] D Tsuruta,[3] CM Ward[1] and R Paus[1] *1 Univ of Manchester, Manchester, United Kingdom, 2 Innsbruck Med Univ, Innsbruck, Austria, 3 Osaka City Univ, Osaka, Japan and 4 Univ of Debrecen, Debrecen, Hungary*

Primary cicatricial alopecias (PCA) like lichen planopilaris (LPP) result in permanent hair loss due to irreversible damage to hair follicle (HF) epithelial stem cells in the bulge. However, stem cell loss does not explain why PCAs typically show scarring. We hypothesized that this may result from epithelial-mesenchymal transition (EMT) of HF epithelial stem cells. During EMT epithelial cells gradually lose their epithelial characteristics and acquire a mesenchymal phenotype. By quantitative immunohistomorphometry, the bulge region of lesional LPP HFs showed a significant increase in the EMT marker proteins, vimentin, and fibronectin, and the EMT-regulative transcription factors, SNAIL, SLUG and TWIST1, compared to healthy HFs. On the other hand, the epithelial marker, E-cadherin, was decreased in the LPP bulge epithelium. Using qRT-PCR, analysis of bulge gene expression (RNA extracts obtained by laser capture microdissection) showed that fibronectin and alpha smooth muscle actin transcription were significantly up-regulated, while E-cadherin expression was down-regulated in the bulge of lesional LPP HFs. In healthy organ-cultured human scalp HFs a cocktail of agents known to promote EMT (interferon-γ, TGFβ1, EGF, peptide A) substantially reduced bulge E-cadherin protein expression, while increasing that of vimentin and SLUG (IHC/IF) after 3-6 days. Moreover, the PPAR-γ agonist, pioglitazone, was capable to prevent experimentally induced EMT development in organ-cultured humans HFs. Taken together, these observations suggest that human HF epithelial stem cells undergo EMT *in situ* during LPP, that EMT can be stimulated even in the bulge of healthy human HFs, and that PPAR-γ agonists counteract bulge EMT. Thus, EMT may explain at least in part the extensive scarring associated with PCAs, and PCA management strategies are needed that inhibit EMT.

667

Electrophysiological and immunohistological characterization of TMEM16A isoforms in human sweat glands

T Ertongur-Fauth,[1] A Brueggemann,[2] C Jost[1] and D Kulichová[3] *1 BRAIN AG, Zwingenberg, Germany, 2 Nanion Technologies, Munich, Germany and 3 Department of Dermatology and Allergology, Ludwig-Maximilians-University Munich, Munich, Germany*

Sweating is an important physiological process to regulate body temperature in humans, and various disorders are associated with dysregulated sweat formation. Primary sweat secretion in human eccrine sweat glands is driven by Ca^{2+}-activated Cl^- channels (CaCC), however, their molecular identity in sweat glands has long remained elusive. Recently, we have provided first evidence that the ion channel TMEM16A contributes to Ca^{2+}-activated Cl^- secretion in human sweat gland epithelial cells. Here, we investigated for the first time localization as well as electrophysiological properties of TMEM16A isoforms in sweat gland cells to further elucidate the function of TMEM16A in sweat secretion. Our gene expression analysis revealed that several *TMEM16A* splice variants including the novel *TMEM16A(acΔe3)*, which lacks the dimerization domain, are expressed in isolated whole eccrine sweat glands. Immunohistological stainings showed that TMEM16A is localized to the apical plasma membrane of cells in the secretory coil of eccrine sweat glands. Chloride flux assays using halide-sensitive YFP as well as high-throughput giga seal patch clamping using SyncroPatch 384PE revealed that TMEM16A mediates Ca^{2+}-activated Cl^- conductance in human NCL-SG3 sweat gland cells. Interestingly, recombinant expression of *TMEM16A* splice variants showed that TMEM16A(acΔe3) is forming a functional CaCC only in NCL-SG3 cells but not in HEK293 cells. Moreover, basal Cl^- currents as well as Cl^- currents induced by internally perfused Ca^{2+} are modified in the novel TMEM16A(acΔe3) isoform compared to canonical TMEM16A(ac). Taken together, our results suggest that cell type-specific transepithelial Cl^- transport in sweat glands is achieved by a complex interaction of different TMEM16A isoforms in conjunction with accessory, sweat gland-specific factors, which provides the opportunity to develop novel therapeutics supplementary to botulinum toxin for treatment of the chronic sweating disorder hyperhidrosis.

668

Hdac1 and hdac2 are required for maintenance and survival of embryonic and adult epidermal stem cells

M LeBoeuf,[1] F Liu,[1] X Zhao,[1] E Olson[2] and S Millar[1] *1 Dermatology, University of Pennsylvania School of Medicine, Philadelphia, PA and 2 University of Texas Southwestern, Dallas, TX*

Histone deacetylases (HDACs) alter gene expression programs through modification of chromatin and transcription factors. Global HDAC inhibitors are approved for treatment of cutaneous T-cell lymphoma, and are currently being tested for therapeutic efficacy in a wide range of malignancies and inflammatory diseases, highlighting the importance of determining precise HDAC functions in normal and diseased tissue. We are using genetic approaches to determine the contributions of HDAC1 and HDAC2 to the embryonic development and postnatal homeostasis of the epidermis. We find that combined deletion of Hdac1 and Hdac2 in embryonic surface ectoderm results in gradually decreased proliferation and increased apoptosis, a dramatic failure of epidermal and hair follicle development, and enhanced expression of the senescence gene p16 and acetylated p53. Interestingly, inducible deletion in adult skin of Hdac1 but not Hdac2 alone causes decreased hair follicle matrix cell proliferation associated with increased matrix-specific expression p16 and p21. Inducible deletion of Hdac1 and Hdac2 together in adult skin causes broad upregulation of p16 throughout hair follicle epithelia and interfollicular epidermis, loss of hair follicle bulge stem cells, and ultimate depletion of deleted epidermal cells, which are outcompeted by residual undeleted cells. These data demonstrate critical requirements for HDAC1 and HDAC2 in epidermal development and regenerative growth, and reveal HDAC1/2 as key survival factors for epidermal stem cells. Our findings identify HDAC1/2 as potential therapeutic targets in epidermal tumors, but further suggest that HDAC inhibition might be ineffective in preventing growth of tumors lacking p16 function.

669

Keratinocytes devoid of DLX3 initiate psoriasis-like inflammation in mice

J Kim,[1] G Nagato,[2] Y Ogawa,[2] MC Udey[2] and M Morasso[1] *1 NIAMS, NIH, Bethesda, MD and 2 NCI, NIH, Bethesda, MD*

The DLX3 homeobox transcription factor is involved in the terminal differentiation of keratinocytes (KCs), and mutations in DLX3 are associated with tricho-dento-osseous syndrome that is characterized by abnormal hair, teeth and bone. We previously reported that epidermal depletion of DLX3 *in vivo* in mice (K14cre;Dlx3$^{kin/f}$ mice; cKO) led to accumulation of IL-17A-producing Vγ4+ γδT cells in skin after postnatal day 14. Based on observations in cKO mice, we hypothesize that DLX3 directly regulates the secretion of inflammatory cytokines by keratinocytes, which leads to the dermal recruitment of immune cells including macrophages and T cells with subsequent IL-17A production. Current studies focus on determining the contribution of the keratinocytes to the inflammatory response and defining the cross talk between the epidermis and dermis in inducible cKO mice (K14ERTcre;DLX3; icKO). To address this, we treated icKO mice with tamoxifen and checked for the development of a psoriasis-like skin phenotype and characterized skin inflammation using flow cytometry analysis of skin single cell suspensions obtained at several time points after tamoxifen treatment (1 and 2 weeks). As early as 1 week after treatment, the skin of icKO mice had increased numbers of immune cells (CD45+ cells) and macrophages (F4/80+ cells) than treated control littermates. RNA-seq analysis will be performed on epidermal and dermal fractions obtained from icKO and control skin to assess cytokine production in DLX3-deleted keratinocytes. Our preliminary data suggests that DLX3 regulates cytokine production by keratinocytes that triggers immune cell recruitment into icKO skin. Further characterization of cytokine networks that modulate inflammation in DLX3-depleted skin may provide insights into human inflammatory skin diseases such as psoriasis.

670

Preventing radiation-induced hair loss by augmenting spontaneous anagen repair through modulating wnt signaling

W Huang,[1] H Chiu[1, 2], C Chan[1, 2] and S Lin[1, 2, 3] *1 Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, 2 Dermatology, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan and 3 Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan*

There is currently a lack of method to prevent radiotherapy-induced alopecia. Whether and how anagen hair follicles attempt to repair themselves in response to ionizing radiation have not been well characterized. We found that there was a dose-dependent effect of ionizing radiation on the severity of dystrophic change of anagen hair follicles. According the severity of dystrophy, anagen hair follicles were able to initiate two spatially and temporally distinct early and late repair activities to restore their structure. We found that bulge stem cells were not activated for the repair. Instead, two precursor cell populations, $K5^+$ and $Lgr5^+$ cells respectively, compensatorily proliferate to restore the anagen hair follicle structure for early and late repair. Molecularly, we found this apoptosis-driven dystrophy was p53-dependent and was accompanied by inhibited wnt signaling. Restoration of wnt signaling preceded the compensatory cell proliferation for the repair attempts. We demonstrated that boosting wnt signaling was able to prevent hair loss by enhancing cell proliferation at an earlier stage to reduce dystrophy. Chemotherapy-induced alopecia could also be attenuated with the same approach. Thus, radiation and chemotherapy-induced alopecia can be prevented by modulating wnt signaling to enhance spontaneous anagen repair.

671

Inducing hair follicle neogenesis with 3 protein factors

S Fan,[2] C Yen,[2] S Pan,[4] Y Chen[3] and S Lin[2, 1] *1 Dermatology, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan, 2 Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, 3 Chemistry, Academia Sinica, Taipei, Taiwan and 4 Institute of Medical Genomics and Proteomics, National Taiwan University, Taipei, Taiwan*

Hair follicle neogenesis depends on the initiation and perpetuation of cross-talk between keratinocytes and dermal cells. We ask whether this epithelial-mesenchymal interaction can be initiated without introducing inductive dermal cells in postnatal life. We found that protein extract from embryonic skin of specific developmental stage was able to induce hair follicle neogenesis both in a full thickness wound and a modified patch assay in mice without the help of inductive dermal papilla cells or newborn dermal cells. Hair follicle neogenesis here was mediated mainly through the effect on fibroblast. When adult fibroblasts, but not keratinocytes, were cultured with the protein extract, they were conferred the ability to induce new hair follicles. To search for the molecular mechanisms involved through phosphoproteomic analysis, we found that insulin/IGF signaling was activated and required for the hair follicle inductivity in adult fibroblasts. Finally, through proteomics analysis, we identified 3 extracellular proteins enriched in embryonic skin that together were required and sufficient to induce hair follicle neogenesis in vivo. Therefore, organ regeneration could be initiated by creating a pro-regeneration environment with defined extracellular factors. Identification of such environmental signals can be incorporated with other approaches to enhance tissue regeneration.

672

Novel diagnostic test predicts mean change in hair counts in female androgenetic alopecia patients treated with topical minoxidil

J McCoy,[1] A Goren,[1] J Roberts[2] and N Desai[2] *1 Research and Development, Applied Biology, Inc., Irvine, CA and 2 Northwest Dermatology Research, Portland, OR*

Topical minoxidil is the only FDA approved drug for the treatment of female androgenetic alopecia. While some patients exhibit great improvement in global photographic assessment, the majority of patients have slight or no response following 6 months of treatment with topical minoxidil. It is thus of great clinical importance to predict treatment outcome prior to initiating minoxidil therapy. Previous studies have demonstrated the correlation between sulfotransferase activity in plucked hair follicles and global photographic assessment of a patient's response to minoxidil therapy. In this study, we aim to assess the ability of the sulfotransferase diagnostic test to predict the mean change in hair counts in female androgenetic alopecia patients treated with topical minoxidil. A small cohort of female pattern hair loss patients were treated with topical minoxidil mono-therapy for 24 weeks. Change in hair counts from baseline as well as a global photographic assessment was conducted. To the best of our knowledge, the minimum percent of hair count change from baseline to be considered a responder to minoxidil has never been formally defined. Based on our analysis of published data from 352 patients, we determined that a patient responding to minoxidil would exhibit a minimum 13.7% increase in hair counts. Utilizing the newly established criterion for a responder based on hair counts, the sulfotransferase diagnostic test in our cohort correctly classified all patients. In addition, the photographic assessment correlated surprisingly well with the hair count assessment (r=1).

673

Alopecia areata is transferred via activated T-lymphocytes

EH Wang,[1] M Khosravi-Maharlooei,[2] R Jalili,[2] R Yu,[2] A Ghahary,[2] J Shapiro[3] and KJ McElwee[1] *1 Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada, 2 Surgery, University of British Columbia, Vancouver, BC, Canada and 3 Dermatology and Skin Science, Vancouver General Hospital, Vancouver, BC, Canada*

The C3H/HeJ mouse strain is one of the most well defined animal models for alopecia areata (AA), a cell-mediated, inflammatory, autoimmune hair loss disease. Both CD4 and CD8 T cells are important for the onset and progression of AA both in humans and rodent models. Currently, grafting full thickness skin from an AA affected C3H/HeJ mouse to healthy recipients is the most practical method to generate larger quantities of AA mice for research. However, this procedure can be cumbersome as it involves invasive procedures and long monitoring time on grafted mice, potentially limiting the accessibility of this model to AA investigators. In our study, we isolated the skin-draining lymph node cells (LNCs) from AA affected C3H/HeJ mice, and cultured six days with the presence of magnetic beads coated with anti-CD3/anti-CD28 crosslinking antibodies, and cytokines, IL2, IL7 and IL15. AA mouse derived LNCs showed similar levels of common CD4+ and CD8+ T-cells subpopulations compared to control LNCs derived from non-AA affected mice, both before and after culture in our initial analysis. However, intra-dermal injection of naïve C3H/HeJ mice with cultured AA LNCs (10 million cells/mouse) induced AA with over 90% success rate, with onset as early as two weeks post-injection. In contrast, control LNCs did not induce AA. Histological analysis showed similar clustering of lymphocytes around the dystrophic anagen hair follicles as observed in other models, however labelled injected cells were not present in the AA lesions. The results indicate an indirect transfer of AA via complex interactions between injected LNCs and the host immune system, and potentially involve special T-cell populations. Our study demonstrates a non-invasive, simplified method to generate large numbers of AA mice that provides a robust alternative to current rodent models for AA research.

674

The role of dermal wnt activation in hair follicle development and carcinogenesis

P Myung,[1] T Yang,[2] P Rompolas[2] and V Greco[2, 1] *1 Dermatology, Yale University, New Haven, CT and 2 Genetics, Yale University, New Haven, CT*

As a conserved regulator of growth, Wnt/β-catenin signaling plays a key role at the interface of development and cancer. During embryonic development, epithelial Wnt activation is one of the earliest events required for hair follicle induction. Similarly in adult skin, Wnt activation by hair follicle epithelial stem cells is critical for hair follicle regeneration. We previously established that dermal niche cells are required for hair follicle regeneration but that forced activation of β-catenin within hair follicle stem cells is sufficient to induce growth independently of native dermal niche cells, suggesting that dermal cells provide important signals upstream of epithelial Wnt activation. In this study, we develop a novel approach to understand how the dermal niche regulates hair follicle growth during development and malignancy. Previous studies showed that dermal Wnt signaling is a critical initiating signal prior to hair follicle induction, as Wnt is activated in the dermis prior to hair follicle induction and is required for subsequent epithelial Wnt activation. However, it is currently unclear how dermal Wnt activation 1) influences dermal cellular behaviors such as proliferation and migration, 2) if and how Wnt-activated dermal cells contribute to formation of the dermal papilla, and 3) how dermal Wnt signaling non-cell autonomously regulates epithelial Wnt activation and organized growth. Using explant cultures coupled with live imaging, we have the tools to directly address these questions. Wnt is activated in the upper dermis prior to hair follicle initiation, and we can track these cells over time during hair follicle induction and dermal papilla formation in skin explant cultures. We also show that dermal Wnt activation is distinctively lacking in basal cell carcinomas, a malignant tumor of the hair follicle germ, and is a possible mechanism for the disorganized and unchecked growth of this tumor. Collectively, this study provides a unifying mechanism that holds important implications for tissue regeneration as well as for basal cell carcinoma.

675

The role of regulatory T-cells in hair follicle cycling

N Ali,[1] H Truong,[1] AK Abbas[2] and MD Rosenblum[1] *1 Dermatology, UCSF, San Francisco, CA and 2 Pathology, UCSF, San Francisco, CA*

Regulatory T cells (Tregs) in skin localize to hair follicles (HF) in both rodents and man. However, it is currently unknown how these cells influence HF biology. We hypothesize that Tregs facilitate normal HF cycling by protecting the HF stem cell niche. To test this hypothesis, we examined HF cycling in mice in which Tregs can be readily deleted in post-natal life (i.e., Foxp3-DTR mice). We found that Tregs become activated upon induction of HF cycling. Depilation-induced HF cycling is absent in Treg depleted mice, suggesting that hair regrowth is dependent upon the presence of Tregs. Examination of the keratinocyte HF stem cell (HFSC) compartment in Treg deleted mice showed a drastic reduction in depilation-induced HFSC proliferation, suggesting a dysregulation in anagen induction. Our ongoing and future work will examine the mechanisms of Treg mediated initiation of HFSC proliferation and concomitant anagen induction during physiological hair cycling. This work will lead to a better understanding of how immune cell populations influence HF biology and how these relationships may be altered in inflammatory hair disorders such as alopecia areata.

676

Enhancing hair follicle regeneration by microthermal injury

Y Wu,[1] S Fan[1] and S Lin[1, 2, 3] *1 Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, 2 Dermatology, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan and 3 Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan*

Identification of methods to enhance anagen entry can be helpful for alopecia. Recently, microthermal injury with nonablative laser has been proposed as a potential treatment for alopecia. However, how the laser affect stem cell activity, hair cycles and the associated side effects have not been well characterized. Here we examine the effects of 1,550-nm fractional laser on hair cycles. The dorsal skin of eight-week-old female C57BL/6 mice with hair follicles in synchronized telogen was shaved and irradiated with a fractional laser with varied beam energies (5-35mJ) and beam densities (500-3500 microthermal zones/cm^2). We found that direct thermal injury to hair follicles could be observed early after irradiation, especially at higher beam energy. Anagen induction in the irradiated skin showed an all-or-non change. Anagen induction and ulcer formation were affected by the combination of beam energy and density. The lowest beam energy of 5mJ failed to promote anagen entry at all beam densities tested. As beam energy increased from 10mJ to 35mJ, we found a decreasing trend of beam density that could induce anagen entry within 7-9 days with activation of hair follicle stem cells. Beam density above the pro-regeneration density could lead to ulcers and scarring followed by anagen entry in adjacent skin. Gene expression analysis showed that transient moderate inflammation was associated with anagen induction and intense prolonged inflammation preceded ulcer formation. To avoid side effects of hair follicle injury and scarring, appropriate combination of beam energy and density is required. Parameters outside the therapeutic window can result in either no anagen promotion or ulcer formation.

677

Apoptotic signals increase during catagen-like changes in hair follicles confirming follicle organ culture's exciting new potential as a human *in vitro* catagen model

HN Rahman,[1] N Farjo,[2] B Farjo[2] and V Randall[1] *1 Biomedical Sciences, University of Bradford, Bradford, United Kingdom and 2 Farjo Medical Institute, Manchester, United Kingdom*

Hair disorders, involving either hair loss or excess, are generally poorly controlled. Altering how long a hair grows (anagen) changes the length of the hair produced, but the factors promoting anagen's end (catagen), are unclear. Understanding these could lead to better therapies, but progress is hampered by the lack of suitable *in vitro* models. Therefore, we aimed to investigate human follicle organ culture as a catagen model by determining whether apoptotic signals were altered in scalp follicles undergoing catagen-like changes. Microdissected scalp hair follicles were observed, measured and photographed daily in organ culture for 4 days. Anagen ending was identified by observing catagen-like changes in the hair bulb microscopically and confirmed histologically. Total RNA was extracted from follicles exhibiting catagen-like changes and matched follicles remaining in anagen (3 pooled samples, each from 5 different people). Differences in gene expression were determined using DNA microarray analysis and confirmed using quantitative real-time PCR. Gene expression of several signaling molecules was altered in catagen-like follicles compared to matched anagen follicles. Levels of growth-promoting FGF10, and its receptor FGFR2, were significantly decreased. In contrast, levels of inhibitory factors TGFB1 and BDNF, and their receptors, TGFBR1 and p75NTR, were significantly increased in catagen-like follicles as reported in mice (Botchkarev et al., 2000). Simultaneous inhibition of stimulatory growth factors and stimulation of inhibitory factors correlated directly with the cessation of hair growth indicating their involvement in entry into catagen. Further investigations using this new human catagen model system should facilitate the development of novel therapeutics targeted at prolonging anagen or initiating catagen for hair disorders.

678

Bimatoprost alters prostaglandinand prostamide synthesis inhuman scalp hair follicles

V Randall,[1] HN Rahman,[1] K Khidhir,[1] O Shalbaf,[1] N Farjo,[2] B Farjo,[2] NJ Poloso,[3] JW Wang,[3] D Woodward[3] and S Picksley[1] *1 Biomedical Sciences, University of Bradford, Bradford, United Kingdom, 2 Farjo Medical Institute, Manchester, United Kingdom and 3 Biological Sciences, Allergan Inc,, Irvine, CA*

Bimatoprost, a prostamide $F_{2\alpha}$ analogue used for glaucoma, stimulates eyelash growth and is under trial for alopecia.Prostamides are recently discovered lipids, closely related to the paracrine regulators, prostaglandins (PG). We previously showed thatscalp follicles contain $PGF_{2\alpha}$and prostamide $F_{2\alpha}$receptors and bimatoprost stimulates rodentand scalp hair growth. Since these receptors may have biological roles in hair follicles, we investigated whether human follicles contain the enzymes that synthesise PGs and prostamidesand whether bimatoprost alters this in scalp follicles in organ culture. Scalp hair follicles were individually microdissected from non-balding scalp skin. RNAwas extracted for enzyme gene expression identification bygene microarray and quantitative real time PCR(3 pooled samples, each from 5 different people) from follicles *ex vivo*or after culture with or without bimatoprost (100nM) for 2 days. Protein expression was examined in frozen scalp skin sections using immunohistochemistry. Follicles expressed all the enzymes necessary to synthesise$PGF_{2\alpha}$and prostamide $F_{2\alpha}$: phospholipase A2 group 2A (PLA2G2A), fatty acid amide hydrolase 1& 2 (FAAH1,2), cyclooxygenase 1& 2 (Cox1, 2),PGF synthase (PGFS),N-acyl phosphatidylethanolamine specific phospholipase D (NAPEPLD), and prostamide/PGF synthase (PM/PGFS). Immunohistochemistry located these enzymes in the hair bulb. Bimatoprost increased prostamide synthesizing enzymes & decreased PG equivalents. Thus, individual scalp hair follicles possess the necessary enzymes for local synthesis of both $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$from phospholipids. Interestingly, bimatoprost reduced PG,but increasedprostamidesynthetic pathways suggesting that these paracrine mediators may have importantnatural roles in hair follicles; further studies may lead to novel therapies for hair disorders.

679

Spatial trans-interactions between lineage-specific gene loci are required for differentiation of the stratified epithelium

A Mardaryev,[1] J Baer,[2] K Poterlowicz,[1] J Rudolf,[1] I Malashchuk,[1] V Rapisarda,[1] V Kumar,[2] A Sharov,[3] J Yarker,[1] M Fessing,[1] T Kohwi-Shigematsu,[4] VA Botchkarev[1, 3] and T Magin[2] *1 Centre for Skin Sciences, University of Bradford, Bradford, United Kingdom, 2 University of Leipzig, Leipzig, Germany, 3 Boston University, Boston, MA and 4 University of California, San Francisco, CA*

During development, the genome of multi-potent stem cells becomes reorganized in 3D nuclear space to establish a proper spatio-temporal regulation of transcription underlying execution of lineage-specific gene expression programs. However, mechanisms coordinating transcription in lineage-specific gene loci located on different chromosomes during cell differentiation are poorly understood. Differentiation of the epidermis is accompanied by tightly-balanced expression of the keratinocyte-specific genes that are clustered in the mammalian genome into several loci including the Keratin type I (KtyI) and type II (KtyII) gene loci located on mouse chromosomes 11 and 15, respectively, as well as the Epidermal Differentiation Complex (EDC) locus located on mouse chromosome 3. Ablation of the entire KtyII gene locus in the epidermis causes dysregulated expression of 37 genes localized in the EDC locus, leading to severe epidermal barrier defects. Chromatin conformation capture (4C) and 3D-FISH assays revealed that *Krt5* gene in the KtyII locus formed a lineage-specific gene interactome containing *Lor* gene from the EDC locus, altered upon KtyII locus ablation. Furthermore, the KtyII locus contains several trans-enhancer elements regulating *Lor* expression. Thus, trans-interactions between lineage-specific loci are essential for coordinated regulation of gene expression, implicating their role in governing epithelial differentiation and function.

680

Polycomb repressive complex maintains epidermal progenitors by repressing key Merkel cell differentiation genes

E Bardot, C Perdigoto and E Ezhkova *Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY*

Merkel cell-neurite complexes are located in touch sensitive areas of the mammalian skin and are involved in recognition of the texture and shape of objects. Merkel cells are essential for these tactile discriminations, as they generate action potentials in response to touch stimuli and induce the firing of innervating afferent nerves. It has been shown that Merkel cells originate from epidermal progenitors, but the cellular and molecular mechanisms of their development are largely unknown. We analyzed Merkel cell differentiation during development and found that it is a temporally regulated maturation process characterized by a sequential activation of Merkel cell specific genes. We uncovered key transcription factors controlling this process and showed that the transcription factor Atoh1 is required for initial Merkel specification. The following maturation steps of Merkel cell differentiation are controlled by cooperative function of the transcription factors Sox2 and Isl1, which physically interact and work to sustain Atoh1 expression. Importantly, Atoh1, Sox2, and Isl1 genes are repressed by the Polycomb repressive complex in epidermal progenitors. This repression is critical to maintain epidermal progenitors in the undifferentiated state as loss-of-Polycomb repression results in Atoh1, Sox2, Isl1 expression and accelerated differentiation of epidermal progenitors to Merkel cells. These findings reveal the presence of a robust transcriptional network required to produce functional Merkel cells that are required for tactile discrimination.

681

Serum response factor (SRF) regulates the development and cyclic regeneration of the hair follicle, and functions in epidermal development in a stage-specific manner

C Lin, A Koppel, A Kiss, L Ma and T Efimova *Medicine/Dermatology, Washington University School of Medicine, St. Louis, MO*

We reported previously that mice conditionally targeted for ablation of the transcription factor SRF in skin epithelia die perinatally due to a compromised barrier function. Conditional mutants with epidermal-specific K14-Cre-mediated *Srf* ablation (Srf^{K14Cre}-cKO) displayed a severely perturbed morphology of embryonic/neonatal epidermal tissue, marked defects in terminal differentiation, and defective hair follicle (HF) development. Additionally, engrafted $Srf^{K14\text{-}Cre}$-cKO skin remained largely hairless. To circumvent lethality, and to enable further examination of the roles for SRF in epidermal and HF homeostasis, we generated and characterized several novel spatiotemporally controlled SRF loss-of-function mouse models. The appearance of skin and hair coat were abnormal in $K5rtTA;TRE\text{-}Cre;Srf^{fl/fl}$($SRF^{K5rtTA}$-cKO) mutants, in which epidermal SRF ablation was induced embryonically. Mutant postnatal day 9 HFs exhibited profound morphological and differentiation defects. Importantly, the number of matrix cells, which represent the transit amplifying cells in the HF, was reduced in the SRF^{K5rtTA}-cKOs. Notably, when SRF deletion was induced after embryonic day (E17.5), the first round of hair development was unaltered, while the next round was perturbed. Analysis of the inducible matrix-specific $Srf^{Msx2rtTA}$-cKOs and the bulge-specific $Srf^{K15CrePR1}$-cKOs suggested a role for SRF in the bulge stem cells and/or the secondary hair germ. Analysis of $Srf^{Megsin\text{-}Cre}$-cKO skin revealed the developmental stage-specific requirement for functional SRF in epidermis. These studies provide further insights into the functional significance of SRF in the development of interfollicular epidermis, and in the development and cyclic regeneration of HFs.

682

Inhibition of JAK-STAT signaling promotes hair growth

S Harel,[1] C Higgins,[1] JE Cerise,[1] JC Chen,[1] Z Dai,[1] R Clynes[1] and A Christiano[1, 2] *1 Dermatology, Columbia University, New York, NY and 2 Genetics & Development, Columbia University, New York, NY*

The JAK-STAT signaling pathway has been implicated in regulation of the immune system, but has not been widely studied in a non-inflammatory context in the skin. Our recent studies discovered that inhibition of the JAK-STAT pathway can both prevent and reverse disease in the mouse model of AA. Unexpectedly, during the course of these studies, we observed that topical treatment with JAK-STAT inhibitors resulted in remarkable and rapid hair growth compared to systemic treatment, suggesting a localized effect of JAK-STAT inhibition on initiation of the hair cycle. This observation invited us to interrogate this property in the context of normal mouse skin, where we found that topical treatment with several JAK-STAT inhibitors resulted in dramatic onset of anagen hair growth within 10 days of treatment. This effect was not dependent on JAK-STAT signaling in lymphocytes in the skin, since treatment-mediated hair growth was observed in T and B cell deficient mouse models. To define the state of JAK-STAT signaling in the hair follicle (HF), we examined the dynamics and localization of signaling components during the hair cycle, and found that activation of Stat3 and Stat5 was observed in stem cell compartments such as the bulge, hair germ and dermal papilla. Functional studies suggest that JAK-STAT inhibition promotes HF stem cell activity, as well as enhances the inductive capacity of dermal papillae in patch assays. To establish relevance in human HFs, we treated grafted human scalp skin with JAK-STAT inhibitors, and showed that inhibition of JAK-STAT signaling is sufficient to stimulate human hair growth. Moreover, JAK-STAT inhibition resulted in the elongation of the hair shaft in human organ culture assays, suggesting that pathway inhibition can both promote anagen induction, as well as prolong an existing anagen. Our findings suggest that blockade of the JAK-STAT pathway represents a new therapeutic target for the promotion of hair growth.

683

Sexual dimorphism in human scalp skin

J Chen[3, 2], GM DeStefano[1] and A Christiano[1, 3] *1 Genetics and Development, Columbia University, New York, NY, 2 Systems Biology, Columbia University, New York, NY and 3 Dermatology, Columbia University, New York, NY*

While it has been established that common mechanisms of hair development and hair loss are influenced by sex hormones and underlying sex-dependent gene expression, little direct work has been done characterizing these effects in adults. Females are slightly more likely to present with alopecia areata (AA) than males, and there is a bias in distribution of clinical severity by gender. Furthermore, there are profound differences in other hair loss phenotypes between women and men, suggesting that a sex-dependent heterogeneity exists in adult scalp skin. We analyzed a cohort of mixed-gender alopecia areata patients and unaffected controls to identify gene expression signatures of AA pathology, segregating the patients into groups corresponding to clinical severity. From these cohorts, we generated a gene expression signature of AA using only autosomal loci and clustered the patients to test signature fidelity. Unexpectedly, we found that the pathological gene expression signatures allowed for highly accurate clustering of all individual samples by gender. Moreover, this result was observed in both the affected (AA patients) and unaffected (control individuals) arms of the cohort, suggesting that adult scalp skin is sexually dimorphic at the molecular level both in normal skin and in AA. A more rigorous analysis of AA patient samples segregated by gender revealed distinct molecular gene expression signatures unique to male and female patients, in addition to the core gene signatures shared among AA patients. Regulatory modeling of gender-segregated AA cohorts suggests the systemic genetic differences in human scalp skin that may contribute to AA susceptibility. These results suggest that fundamental sex-linked molecular differences exist in make vs female scalp skin and must be considered in the study of etiology of different forms of hair loss, and gender-specific biomarker design for use in clinical response. Computational models may provide an understanding of these complex molecular behaviors in human skin.

684

Prostaglandin D2 (PGD2) enhances testosterone metabolism in primary human keratinocytes possibly via upregulation of aldo-keto reductase 1C3 (AKR1C3) expression

A Mantel,[1] AP Pentland[2] and M Katdare[1, 3] *1 Hampton University Skin of Color Research Institute, Hampton University, Hampton, VA, 2 Dermatology, University of Rochester, Rochester, NY and 3 Dermatology, Eastern Virginia Medical School, Norfolk, VA*

Prostaglandin D2 (PGD2) is a lipid mediator that has been mostly implicated in promoting allergic responses of the skin, and more recently shown to promote androgenic alopecia and inhibit wound-induced hair follicle neogenesis. Our previous data showed that human keratinocytes treated with PGD2 upregulate the expression of Aldo-Keto Reductase 1C3 (AKR1C3), an enzyme that has been shown to convert the weak androgen androstenedione (AD) to the more potent steroid, testosterone. The role of testosterone and its reduced product, dihydrotestosterone, as drivers of androgenic alopecia is well established. The local concentration of testosterone in the skin depends in part on the expression level of various testosterone-synthesizing enzymes, thus, any chronic dysregulation of their expression may indirectly promote pathology. We hypothesized that AKR1C3 upregulation in PGD2-treated keratinocytes increases their capacity to generate testosterone from AD. Primary human keratinocytes were treated with PGD2, PGE2 or vehicle alone for 48 hours followed by exposure to AD for 6 and 24 hours. Spent-medium was collected, and testosterone was assessed by ELISA and data normalized to total protein. Western blot analysis confirmed upregulation of AKR1C3 expression in PGD2 but not PGE2-treated keratinocytes. Preliminary results suggest that 6 hours post AD treatment testosterone was detectable only in the spent-medium of PGD2-treated keratinocytes. By 24 hours post AD treatment, PGD2-treated keratinocytes generated significantly higher levels of testosterone (range of 3-5 folds) compared with PGE2 and DMSO treated groups. Our data suggest that PGD2 may indirectly regulate androgen metabolism in human keratinocytes, possibly via upregulation of AKR1C3. Experiments utilizing AKR1C3 specific inhibitors and siRNA are currently being conducted to validate this assumption.

685

Wnt/β-catenin signaling marks self-renewing stem cells in multiple epithelial tissues

M Xu,[1] J Horrell,[1] H Gochnauer,[1] J Cui,[1] M Snitow,[2] T Peng,[2] E Morrisey[2] and S Millar[1, 3] *1 Department of Dermatology, University of Pennsylvania, Philadelphia, PA, 2 Department of Medicine, University of Pennsylvania, Philadelphia, PA and 3 Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA*

Mutation of the human Wnt ligand WNT10A is associated with adolescent onset of a range of ectodermal defects including thinning hair, smooth tongue, palmoplantar keratoderma and sweating abnormalities. Mice lacking Wnt10a provide a faithful model for the human disease and display decreased signaling through the Wnt/β-catenin pathway, and reduced proliferation of epithelial progenitor cells in hair follicles, tongue filiform papillae, taste buds, palmoplantar epidermis, and sweat gland ducts. To determine whether Wnt/β-catenin signaling marks self-renewing stem cells in these organs we used mice in which tamoxifen-inducible Cre recombinase is knocked into the locus for Axin2, a ubiquitous direct Wnt target gene, in combination with fluorescent Cre reporter transgenes that permit lineage tracing of Wnt-active cells. Wnt-responsive basal cells labeled in adult life by transient Cre activation gave rise to differentiated cells in filiform papillae, taste buds, and palmoplantar epidermis, and were maintained over many cycles of natural epithelial regeneration, indicating that they are able to self-renew. Axin2-active basal cells in adult sweat gland ducts were able to self-renew, but did not give rise to luminal cells, suggesting that a separate stem cell population maintains luminal cells. These data indicate that stem cells in multiple adult epithelia are activated by Wnt/β-catenin signaling, identify Wnt10a as a critical ligand for epithelial progenitor cell proliferation, and suggest downstream activation of the Wnt/β-catenin pathway as a potential means to treat hair loss and palmoplantar defects in WNT10A patients.

686

Alopecia areata and atopic dermatitis share common Th2 and IL-23 inflammatory pathways, with implications for targeted therapeutics

S Noda[1, 2], A Shroff,[2] Y Mansouri,[2] JB Fuentes-Duculan,[1] A Shemer,[3] M Suarez-Farinas,[1] JG Krueger[1] and E Guttman-Yassky[1, 2] *1 Laboratory of Investigative Dermatology, The Rockefeller University, New York, NY, 2 Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, NY and 3 Department of Dermatology, The Chaim Sheba Medical Center, Tel-Hashomer, Israel*

Alopecia areata (AA) is an autoimmune disease with a 2% lifetime prevalence. Current treatments lack efficacy and specificity, with a large need for development of targeted therapeutics based on AA-specific immune pathways. AA often coexists with other inflammatory skin diseases, particularly with atopic dermatitis (AD) in ~40%, potentially alluding for common immune pathways between AA and AD. We sought to characterize the AA skin phenotype and compare with polar Th2 (AD) and Th17/Th1 (psoriasis) diseases, using lesional and non-lesional samples from 22 AA, 13 AD, 10 psoriasis, and normal scalp from 3 healthy individuals. Genomic profiling was performed using RT-PCR and gene-arrays. While the highest activation of Th1 pathway genes (CXCL9, CXL10, STAT1) was seen in the psoriasis phenotype (lesional vs non-lesional), significant inductions were also seen in AD, followed by AA phenotypes. A similar pattern of Th2 (IL-13, CCL18, CCL22, CCL5) and JAK pathway genes activation was seen in AA and AD ($p<0.05$ for all), albeit generally higher for AD. Significant inductions of IL-23 (p40 and p19), and IL-12RB1 were observed in all three diseases ($p<0.05$, more than 3 fold changes/FCHs for p40 for all). IL-15 was induced in both AA and AD while IL-2 was induced only in AA (2 FCHs); $p<0.05$ for all. AA showed a lack of Th17/Th22 (IL-17A, CCL20, PI3, S100A7/8/12) immune polarization compared with both diseases, and particularly versus psoriasis. Overall, AA shows similar Th1, Th2, and IL-23 polarization to AD, with lack of Th17/Th22 skewing. Our data suggest the potential consideration of anti Th2, IL-23p40/p19, and IL-2/IL-15 targeting for AA. Clinical trials with selective antagonists are required to dissect key pathogenic pathways, similar to psoriasis and now also AD.

687

***Gorab* is essential for dermal papilla cells to respond to hedgehog signals during hair follicle formation**

Y Liu,[1] ER Snedecor,[2] Y Choi,[2] N Yang,[2] X Zhang,[1] Y Xu,[1] Y Han,[1] EC Jones,[3] KR Shroyer,[2] RA Clark,[3] L Zhang,[1] C Qin[1] and J Chen[2, 3] *1 Institute of Laboratory Animal Science, Beijing, China, 2 Pathology, Stony Brook University, Stony Brook, NY and 3 Dermatology, Stony Brook University, Stony Brook, NY*

GORAB is a Golgi-associated protein. Autosomal recessive mutations in the *GORAB* gene are responsible for the development of geroderma osteodysplasticum (GO), a congenital premature aging disorder characterized by wrinkly skin and osteoporosis. The molecular mechanism underlying GO is unknown. In this study we investigated the function of GORAB during skin morphogenesis. We found that *Gorab* is essential for hair follicle morphogenesis and that disrupting the expression of the *Gorab* gene caused dermal papilla cells to be unable to respond to hedgehog (Hh) signals. Furthermore, we found that the formation of primary cilium, the subcellular organelle responsible for processing Hh signals, was disrupted in mutant dermal papilla cells. Interestingly, these effects were specifically restricted to dermal papilla cells but not mutant follicular keratinocytes. Examination of resident Golgi proteins revealed that the expression and localization of these proteins were unaltered in *Gorab*-deficient cells. Thus, data obtained from this study not only suggested that GORAB may participate in Hh signaling by facilitating the trafficking of ciliogenic proteins at the Golgi levels but also that abnormal functions of GORAB and other Golgi-associated proteins may result in the development of aging-associated phenotypes, such as hair loss.

688

The distal end of the arrector pili muscle is a potential epidermal stem cell niche

N Torkamani,[2] N Rufaut[1, 2], L Jones[1] and R Sinclair[1, 2] *1 Dermatology, Epworth Hospital, Richmond, VIC, Australia and 2 Dermatology, University of Melbourne, Melbourne, VIC, Australia*

Inconsistent with the view that epidermal stem cells are randomly spread along the basal layer of the epidermis, we found that cells expressing epidermal stem cell markers exist exclusively in direct connection with the distal end of the arrector pili muscle (APM). The distal ends of the arrector pili muscle were identified by immunofluorescent staining for α5 integrin and phalloidin. The epidermal cells that expressed stem cell markers such as Cytokeratin 15 (K15), Melanoma-associated chondroitin sulfate (MCSP), and α6 integrin consisted of a subpopulation of basal keratinocytes located at the attachment site of the APM to the epidermal basement membrane. The distal APM may contribute to the formation of a niche for epidermal stem cells, as the proximal muscle does for follicular stem cells. These cells are hypothesized to participate directly in epidermal renewal and homeostasis, and also indirectly in wound healing, through communication with the hair follicle bulge epithelial stem cell population through the APM. Our findings, plus a re-evaluation of the literature, support the hierarchical model of inter-follicular epidermal stem cells. The co-location of epidermal stem cells with the distal APM provides insights into epidermal control and the possible involvement of epidermal stem cells in non-melanoma skin carcinogenesis.

689

Wnt signaling controls the reversible differentiation of melanocyte stem cells during their self-renewal

Q Sun,[1] H Hu,[1] M Takeo,[1] W Lee,[1] MM Taketo[2] and M Ito[1] *1 Dermatology, New York University Langone Medical Center, New York, NY and 2 Kyoto University, Kyoto, Japan*

A hallmark of adult stem cells is their self-renewal. However, despite the presence of stem cells, most tissues functionally deteriorate over time rather than remain ageless. Here, we reveal that adult melanocyte stem cells synchronously activate a differentiation program dictated by their epithelial niche each time McSCs are employed for tissue regeneration. Prior to producing mature melanocytes, McSCs undergo phenotypic differentiation and subsequently revert back to undifferentiated McSCs while mature melanocytes are terminally differentiated. This ability of McSCs to oscillate between distinct differentiated and undifferentiated/stem cell states is due to the dual function of temporal Wnt activation in driving McSC differentiation as well as self-renewal. In aged mice, phenotypically differentiated McSCs fail to terminate Wnt signaling, leading to sustained Wnt activation and failure to return to their undifferentiated state, ultimately resulting in their loss. These results propose a model in which adult stem cells undergo partial differentiation during a regenerative response instead of maintaining a static undifferentiated stem cell pool, thus raising the risk of stem cell depletion.

690

MHC genes associated with alopecia areata exhibit diverse and complex expression patterns during hair follicle development in mice

A Awgulewitsch[1] and JP Sundberg[2] *1 Medicine and Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC and 2 The Jackson Laboratory, Bar Harbor, ME*

Members of the major histocompatibility complex (MHC) in mouse and human have been implicated as key components of immune privilege of the hair follicle and its putative collapse in alopecia areata (AA). However, little is known about the transcriptional expression patterns of MHC genes that have been associated with AA, either by human genome-wide association studies in AA or transcriptome analysis of skin from the AA mouse model C3H/HeJ in which AA was induced by grafting full-thickness AA-affected skin. Most conspicuously, this includes *H2-Aa* (human HLA-DQA1) and *Ly6g6c* (*LY6G6C*) in both mouse and human, and *H2-T24* in mice, whose expression levels are up-regulated in AA skin of C3H/HeJ mice compared to uninvolved skin of the same strain. The purpose of this study was to determine these genes' histological transcriptional expression patterns by *in situ* hybridization analysis of postnatal skin from C3H/HeJ and FVB/NTac mice. The data show diverse and complex expression patterns that undergo dynamic changes during hair follicle morphogenesis. The expression in various cellular compartments of the hair follicle, dermis, and epidermis of both C3H/HeJ and FVB/NTac mice suggests involvement of multiple lineages in the autoimmune response of AA, as well as in other skin diseases with an autoimmune component.

691

3D cultures of hair follicles on Gelfoam® promote functional recovery of severed peripheral nerves and the spinal cord when transplanted to the injury site

W Cao[1, 2, 3], L Li,[1] S Mii,[4] Y Amoh,[4] F Liu[2] and RM Hoffman[1, 3] *1 AntiCancer Inc., San Diego, CA, 2 Dept. of Anatomy, Second Military Medical University, Shanghai, China, 3 Dept. of Surgery, University of California San Diego, San Diego, CA and 4 Dept. of Dermatology, Kitasato University, Kanagawa, Japan*

We have previously reported that nestin-expressing hair-follicle pluripotent stem cells promote peripheral nerve and spinal cord regeneration. In order to find a more effective and practical way to use the pluripotent hair follicle stem cells for nerve and spinal cord regeneration, 3D Gelfoam® hair follicle cultures, in which the hair-follicle sensory nerve is growing from nestin-expressing stem cells, were transplanted to the injury site of severed sciatic nerves and spinal cords. Nestin-expressing mouse-whisker hair follicles, cultured in vitro for 3 weeks on Gelfoam®, were transplanted along with the Gelfoam® as a scaffold, at the injury site of transected sciatic nerves in nude mice. Within 2 weeks, hair follicle-Gelfoam® transplantation conferred sciatic nerve function, determined by a walking track analysis footprint test, better than transplantation of Gelfoam®-only. Furthermore, balance walking on a narrow cylinder at 10 weeks after transaction, showed that hair follicle Gelfoam® culture transplantation conferred better body balance and locomotion than mice treated with Gelfoam® only or untreated mice, in which the latter two groups of mice had frequent limping and poor balance. Hair follicle-Gelfoam® culture was also transplanted to mice with partial spinal cord severence at T10. Hair follicle-Gelfoam® culture transplantation achieved significantly greater locomotor recovery than Gelfoam®-only or control animals, as measured by Basso Mouse Scale (BMS) quantitative walking analysis. These results suggest that transplantation of the 3D cultures of the hair follicle on Gelfoam® promotes perpheral nerve and spinal cord regeneration. The 3D Gelfoam® culture system of hair follicles provides an accessible, effective, stem-cell scaffold for nerve and spinal cord injury repair and regeneration, which should have high clinical potential.

692

Molecular diagnostics in differentiation of segmental overgrowth syndromes

L Youssefian[1, 2], H Vahidnezhad[1, 2, 3], T Baghdadi,[4] A Ghaznavi,[4] Q Li,[2] M Tabrizi[1] and J Uitto[2] *1 Tehran University of Medical Sciences, Tehran, Iran (the Islamic Republic of), 2 Thomas Jefferson University, Philadelphia, PA, 3 Pasteur Institute of Iran, Tehran, Iran (the Islamic Republic of) and 4 Imam Khomeini Hospital, Tehran, Iran (the Islamic Republic of)*

Segmental overgrowth syndromes, a spectrum of disorders with vascular, cutaneous and skeletal abnormalities, are sporadic, non-hereditary disorders, the prototype being the Proteus syndrome (PS), caused by heterozygous activating mutations in the *AKT1* gene. In differential diagnosis with PS is the CLOVES syndrome, with overlapping features of fibroadipose hyperplasia (FH), characterized by segmental overgrowth of fibrous and adipose tissue and bone, belonging to PIK3CA-related Overgrowth Spectrum. Here we describe a patient with segmental hypertrophy initially considered to have PS, but the mutation analysis confirmed the diagnosis of FH. The patient at the age of 3 yrs was noted to have congenital progressive overgrowth in her right lower limb, histologically a mixture of adipose and fibrous tissue in mosaic distribution. DNA was isolated from leukocytes and from affected and unaffected areas of skin. Sequencing of *PIK3CA* revealed a mutation c.1624G→A (p.Glu542Lys) in the affected tissue while normal skin and blood were negative. This sequence variant is not present in the SNP databases, and bioinformatics analyses predicted it be "Damaging". The Glu542 residue, conserved through evolution, changes the charge and size of this residue shown to be involved in multimeric assembly of the protein complex. These conformational and functional changes are accompanied by activation of PI3K leading to cell proliferation. Thus, this amino acid substitution is a somatic mutation in a mosaic cell population, consistent with the diagnosis of FH. This diagnosis was further supported by exclusion of PS diagnosis, since the recurrent, and the only mutation disclosed in PS, c49G→A (p.Glu17Lys) in *AKT1*, was not present. This case illustrates the role of mutation analysis in differential diagnosis of tissue overgrowth syndromes.

693

Studying hair cycle clock with the aid of multi-scale diffusion-based mathematical modeling

J Oh, Q Wang, Q Nie and M Plikus *University of California, Irvine, Irvine, CA*

Hair follicle (HF) is the model system of choice for studying mechanisms of regeneration. Each HF features a prominent stem cell compartment and a tractable regeneration cycle, consisting of the anagen, catagen, and telogen phases. To-date, the fundamental mechanism underlying the timing of hair growth, aka "hair cycle clock" remains largely unknown. One possibility is that the hair cycle clock is composed of two or more activator and inhibitor signaling pathway pairs and that key hair cycle phase transitions occur at certain cumulative thresholds for these pathway activities. Herein we developed a mathematical model that accounts for natural HF geometry and cell dynamics. Incorporating activator/inhibitor signals in the context of this model produces stable periodicity and excitability – hallmark features of the natural hair cycle. In the context of our model, activator/inhibitor signals were predicted to have opposing effects on anagen and telogen phase periodicity. Increasing activator levels was predicted to shorten telogen and lengthen anagen. The inverse effects were modeled for an inhibitor. Here, we focused on anagen phase length and validated these predictions for BMP/WNT signaling pair. Using mouse models we show that decreasing inhibitory BMP signaling leads to the production of longer hairs, thus indicating a longer anagen. We also demonstrate that decreasing activating WNT signaling in mutant mice results in shorter hairs. Finally, we showed that some hair types were the most sensitive to changes in BMP levels than others, suggesting differential effects of BMP modulation. Simulations of this phenomenon suggests that changes in just one background model parameter is sufficient to recapitulate differential sensitivity of hair types to the same net change in the activator/inhibitor signaling levels. Taken together, we provide the first example of a diffusion-based mathematical model that accounts for realistic changes in HF geometry, displays stable periodicity and excitability. It creates a novel opportunity for studying the hair cycle clock mechanism using Systems Biology approach.

694

The LINC complex promotes keratinocyte cell-cell adhesion and hair follicle structure

R Stewart, A Zubek, KA Rosowski, M King and V Horsley *Yale University, New Haven, CT*

Robust cell adhesions afford the epidermis the ability to withstand mechanical stress. Integration of the cytoskeletal network and cell adhesions throughout mammalian tissues is thought to occur through nuclear envelope-spanning LINC complexes, which are critical for nuclear migration, positioning, and anchorage; however, these functions in the skin are not well understood. Here, we describe a new role for the LINC complex protein Sun2 in maintenance of cell-cell adhesion in murine keratinocytes and hair follicles. Sun2-deficient mice displayed follicular and hair shaft defects during the first hair cycle, leading to alopecia that resolved by the second hair cycle. A compensatory increase in Sun1 expression in *Sun2-/-* hair follicles may explain the restoration of hair follicle structure by rescuing LINC complex function. Ultrastructural study of *Sun2-/-* hair follicles revealed imbalanced desmosome density across trichocyte layers, altered desmosome morphology and widened intercellular gaps. *In vitro*, *Sun2-/-* keratinocytes displayed morphologically normal desmosome- and adherens junction-based contacts but adhesion was functionally defective in response to mechanical stress. In addition, *Sun2-/-* cells exhibited aberrant nuclear positioning in response to adhesion formation, with excessive nuclear movement toward cell-cell adhesions. This phenotype was recapitulated by pharmacologic inhibition of microtubule (MT) polymerization and mitigated by actin depolymerization. Further, *Sun2-/-* cells displayed defective reorganization of the MT network upon adhesion formation. Overall, our data suggest Sun2-mediated nucleo-cytoskeletal integration is critical in the function of intercellular adhesions both *in vivo* and *in vitro*. Further, MT and actin networks likely oppose one another to position nuclei in response to adhesion, and strong cell-cell adhesion requires coordinated remodeling of the cytoskeleton at the nuclear envelope and cell-cell adhesions necessary for the mechanical integrity of the epidermis and hair follicle.

695

Rapid hair cycle pattern breakdown during mouse development revealed with the aid of mathematical modeling

J Oh, Q Wang, Q Nie and M Plikus *University of California, Irvine, Irvine, CA*

Recognized for its periodicity, excitability, and patterning, the hair follicle (HF) is becoming a preferred biological system for the mathematical modeling of regeneration. Cyclic growth of HFs is regulated both by signaling interactions within the HF (signaling micro-environment) and long-range signals between neighboring HFs and other skin cells (macro-environment). Herein, we developed a mathematical model based on the molecular dynamics of parallel activator/inhibitor pathways, where both single HF and population level behaviors emerge naturally upon scaling. We modeled the phenomenon of age-dependent hair cycle pattern breakdown, wherein highly synchronous hair growth in the first two cycles is thought to become replaced by the asymmetric hair growth waves in the third cycle. Surprisingly, our modeling shows that the breakdown in the hair growth symmetry requires approximately ten hair cycles, far more than the observable two cycles. Additional simulations predicted two new requirements for the rapid hair growth pattern evolution: (i) hair growth asynchrony must already exist during the first, morphogenetic hair cycle; and (ii) two or more HF populations with distinct hair cycle parameters must interact with one another. Next, we performed a detailed hair growth pattern analysis during the first two hair cycles. Indeed, we found previously unrecognized spatial-temporal wave of hair morphogenesis. Furthermore, we identified previously unknown interactions between anatomically distinct HF populations at the onset of the second anagen. Taken together, here we applied a Systems Biology approach to reveal previously unrecognized hair cycle dynamics that contribute to rapid hair growth pattern evolution in mouse skin. Our findings challenge the prevailing view that the first two hair cycles as been synchronous. They have important implications for designing and interpreting future hair cycle experiments.

696

Genetic determinants of eccrine sweat gland density in the mouse

B Morgan[1, 2], Y Kamberov[3] and C Tabin[3] *1 Dermatology, Harvard Medical School, Boston, MA, 2 Dermatology, Massachusetts General Hospital, Boston, MA and 3 Genetics, Harvard Medical School, Boston, MA*

When compared to other mammals, human skin exhibits a high density of eccrine sweat glands interspersed among diminished hair follicles. These traits are thought to have played a critical role in human evolution by providing increased thermoregulatory capacity. Understanding the genetic basis of these changes will be critical to the improved repair of human skin in vivo or its generation in vitro. Given the dearth of information on eccrine sweat gland development, we employed Quantitative Trait Linkage analysis to identify loci that alter the relative density of eccrine glands and hair follicles in the interfootpad region of the mouse paw where these two appendages are interspersed as they are in human skin. This approach identified several regions with strong effect size. Within the strongest of these, allele specific expression comparisons in F1 skin identified candidate genes to regulate eccrine gland and hair follicle density. Functional analysis revealed that modification of the expression of a single gene in the region is sufficient to reciprocally alter the relative density of eccrine glands and hair follicles. This approach identifies genetic pathways that regulate ecrrine sweat gland density in the mouse and are candidates to drive the unique characters of human skin.